X

SOME CONTRIBUTIONS TO THE STUDY OF THE PHYSIOLOGY OF ALLANTOÏN.

THESIS

FOR THE DEGREE OF M. D.

Presented by

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Since allantoïn was first discovered in the allantoic fluid by Vauquelin[1] in 1840, a great deal of work has been done of chemical and physiological significance.

Chemistry.

Allantoïn is represented by the formula $C_4 H_6 N_4 O_3$ and is now known to be a diureide related to uric acid $C_5 H_4 N_4 O_3$ but with another acid radicle as the linking band.

It crystallises in large monoclinic crystals which are hexagonal in shape and form sheaves and rosettes.

In reaction allantoïn is neutral. It dissolves in cold water with difficulty, being soluble in only 160 parts of cold water at 20° according to Liebig und Wöhler[2], and in 186 parts according to Schulze und Barbieri.[3] It is more soluble in hot water, requiring only 30 parts, and can be dissolved in absolute alcohol - more readily when hot. It can also be dissolved by the carbonates of the alkalis, but not by aether.

Allantoïn melts at 210°- 220°C.

It can combine with bases to form salts and with silver forms a compound known as allantoïn silver $C_4 H_5 Ag N_4 O_3$ precipitated after the addition of ammonia.

It reduces Fehling's solution and is one of the products of the oxidation of uric acid, and by further oxidation gives urea.

It may be prepared by the careful oxidation of uric acid by permanganate of potassium in neutral or alkaline solution, or by ferrocyanide of potassium, plumbic oxide, ozone, or cupric oxide. By oxidation with alkalis, uric acid is converted into allantoïn and carbonic acid.

Physiology.

Allantoïn was first thought to be a constituent of foetal urine and foetal metabolism. Subsequently it was found in the urine of adult man by Ziegler und Hermann[4]; and in that of cats, dogs, and rabbits by Meissner.[5] Pouchet[6] also found it in the urine of man and in greater amount in the urine of pregnant women, as well as in diabetes insipidus and convulsive hysteria.

Attention was more recently drawn to it by Salkowski[7] in 1876, demonstrating its presence after feeding uric acid to dogs when four grammes were given on each of two successive days, and 1.42 grammes of allantoïn were recovered, and later the same investigator found it in crystals in the urine of dogs after thymus feeding, and also after pancreas feeding.

Mendel and Brown[8] obtained a considerable yield of allantoïn after feeding uric acid to cats, but more recently Poduschka[9] has failed to find it after feeding uric acid to dogs.

Minkowski and Cohn[10] discovered independently that allantoïn may be excreted in the dog as an end-product in metabolic processes which give rise to uric acid elimination after thymus feeding.

An increased output of oxalic acid was obtained by Luzzato[11] after feeding allantoïn to rabbits, and he points out that in view of the ready oxidation of oxalic acid in the body, it may easily escape elimination as such by being destroyed. The introduction of nucleic acid either intravenously, intraperitoneally, or subcutaneously, has been shewn by Mendel, Underhill, and White,[12] to be followed by an elimination of allantoïn in the dog and cat. Mendel[13] also found that he could produce an increase in the amount of uric acid and a considerable increase of allantoïn after rectal injections of thymus extract, and an increase of allantoïn in cats after being fed with vegetable nucleo-proteid. Mochizucki[14] has shewn that the administration of thymus gland substance per rectum is also followed by a typical increase in uric acid excretion in man.

The Effect of Drugs on Allantoïn Excretion:-

The effect of drugs on allantoïn excretion has also been investigated.

Lithium Urate :- Lafayette, Underhill, and White[15] have shewn that allantoïn could be produced by the injection of uric acid as lithium urate.

Sulphonal:- Dogs subjected to sulphonal intoxication excrete considerably less allantoïn after urate injections than more normal dogs. Paton and Eason[16] have interfered with hepatic metabolism by the administration of sulphonal. They have found that by this drug they could diminish the proportion of waste nitrogen elaborated into urea. Lafayette, Underhill, and White[17] have studied allantoïn excretion in dogs intoxicated with sulphonal, the animals being fed on pancreas, and the disappearance of allantoïn from the urine after pancreas feeding during sulphonal intoxication was constantly observed.

Hydrazine poisoning:- Pohl[18] failed to find allantoïn in the tissues of the normal dog but in hydrazine poisoning it appears in the liver and in traces elsewhere. On a few hours autolysis of the organs allantoïn appeared chiefly in the intestinal mucous membrane and in the liver.

Hydroxylamine:- Hydroxylamine can produce allantoïn, but it does not appear with arsenic

or phosphorus. (Pohl)[18]

Diamido sulphate:- In dogs diamido sulphate injected in doses of .05 grm. per kilo, causes coma and death with the presence of allantoïn in the urine. (Borissow)[19]

Hydrazine sulphate:- Hydrazine sulphate does not cause the production of allantoïn.

Persulphate of Ammonium:- Persulphate of ammonium can cause the conversion of uric acid to allantoïn with oxalic acid and urea. This change takes place outside the body. (Hugonneg)[20]

Lactate of Ammonium:- Lactate of ammonium causes no increase of uric acid in dogs, but thymus does, although not so much as in man. (Minkowski)[21]

The question cannot yet be considered as settled - "From what is allantoïn formed."

It may be a derivative of the metabolism of nucleins or it may be simply formed from purin bodies.

The nucleins have been shewn to split up into phosphoric acid and purin bodies of which uric acid is the chief in man.

One great difficulty in deciding the question is the fact that allantoïn, like other purins is probably in part converted into urea in the animal body and thus it is somewhat difficult to correctly

interpret alterations in the amount of the substance excreted.

If allantoïn were formed from the nucleins, one would expect the same yield of allantoïn when a dog is fed on raw, as when fed on boiled, thymus gland, and less when the animal is fed upon an extract of the thymus gland containing none of the nucleins, but containing purins. But there is always the possibility that the thymus gland may yield material of the nature of an internal secretion, which in young animals so modifies the metabolism as to allow of the formation and excretion of allantoïn.

The influence of feeding with pancreas, which is nearly as rich in nucleins as the thymus gland, is also referred to in the following experiments.

Method of Experiment.

To endeavour to throw light upon these questions, a quantitative estimation of the allantoïn in the urine was made after feeding the dogs on a diet of oatmeal porridge and milk to obtain nitrogenous balance and then upon various diets - the nitrogenous balance being again returned to with each change of diet.

During the experiments the dogs were kept in zinc cages with sloping floors, and the urine was received in suitable vessels underneath.

The examination was made, in each case, upon the urine of twenty-four.

To ensure accuracy in results, the amount of fat with each thymus gland was estimated by Soxhlet's method, so that the quantity of gland substance as such might be known by deducting the amount of fat.

The extract of thymus gland was prepared by boiling the minced gland for three hours to be sure that the internal portions of the gland were thoroughly cooked and then the whole mass was filtered through calico. This filtrate was then passed through a filter paper with the aid of a Sprengel pump and the resulting filtrate constituted the "extract of thymus gland."

Method of Analysis.

1. Loewi's[22] method - a method which depends upon the precipitation of the nitrogenous compounds by means of mercurous nitrate without precipitating allantoïn, is unsatisfactory, owing to the fact that mercurous nitrate, of too acid, or if impure (containing mercuric nitrate), will also precipitate allantoïn.

2. The second method - that of Moscatelli,[23] consists in the precipitation of allantoïn with mercuric nitrate.

The subsequent washing of the precipitate was

found to result in considerable loss. Another cause of loss is the addition of too much ammonia thereby setting free the allantoïn nitrogen.

3. The next method adopted was that of Poduschka[24] of which the following is a description: - 50 or 100 c.c. of the urine are precipitated with basic lead acetate.

The excess of lead is then removed from a definite volume of the filtrate by concentrated sodium sulphate solution.

To a definite volume of the second filtrate 5 - 10% silver nitrate solution is added.

This is then filtered and the precipitate is rejected.

Dilute ammonia is then added drop by drop (1% solution) to the new filtrate.

The allantoïn silver is now precipitated, and washed thoroughly with 1% sodium sulphate solution until free from ammonia.

The allantoïn is then estimated by Kjeldahl's method.

The difficulty with this method is to render the precipitate free from ammonia.

If the precipitate be not free from ammonia then the nitrogen of the added ammonia would be estimated as allantoïn nitrogen. To render the precipitate ammonia free it must be washed with 1% sodium sulphate solution. To decide when the

precipitate was free from ammonia the absence of the alkaline reaction to red litmus paper was taken as the indicator. By this time the results obtained with pure allantoïn gave only a small fractional return, viz., 1.1%, 1.4%, and .8%, i.e., an average of 1.1% was recovered. With this washing the precipitate was visibly disappearing. The filtrate resulting from the washing of the precipitate was subjected to the following tests:-

Hydrochloric acid yielded a white precipitate soluble in ammonia.

Potassium hydrate gave a brown precipitate insoluble in excess.

Potassium iodide gave a pale yellow precipitate insoluble in nitric acid.

Potassium chromate produced a red precipitate soluble in dilute nitric acid.

These reactions indicate the presence of a silver salt.

As the washing of the precipitate was continued these reactions became less pronounced. To demonstrate the appearance of allantoïn in the filtrate .2 grm. of allantoïn (Merck) was placed in a desiccator over sulphuric acid until no further loss in weight was noticed (one week). When of constant weight .2 grm. had been reduced

to .196 grm. allantoïn. This was then utilised for a "recovery experiment". The precipitate of allantoïn silver was then washed with 1% sodium sulphate in boiled and cooled distilled water as tap water gives, when carefully tested, an alkaline reaction.

After being washed with the solution the precipitate was tested and gave an alkaline reaction. The process of washing was discontinued, although the precipitate was not yet ammonia free on the assumption that the silver allantoïn must have appeared in the filtrate in sufficient amount to indicate its presence.

This filtrate must contain the ammonia added to precipitate the allantoïn silver and this if estimated as allantoïn nitrogen would render the "recovery experiment" fallacious. To overcome this source of error the nitrogen of the ammonia was set free by means of oxide of magnesium at a temperature of 60°c until the fumes no longer reacted alkaline.

The amount of allantoïn in the filtrate was then found to be 13.2% of the original precipitate. Had the precipitate been washed until free of ammonia a greater loss would have occurred, as indicated by the experiments previously described when only 1.1% remained of the precipitate

The presence of silver allantoïn having been demonstrated in the filtrate, and the filtrate being rejected in Poduschka's method, a loss in the amount of silver allantoïn recovered by means of the precipitate is the result.

It would therefore appear that the more one washes the precipitate, the smaller is the result, and the less one washes the precipitate, the nearer it approaches to a correct result, although not free from ammonia.

To overcome this difficulty, the following modification of Poduschka's method was devised:-

The filter paper with its unwashed precipitate was placed in an Erlenmeyer's flask with a pinch of oxide of magnesium and a little water, over the steam-bath at 60°c, until the steam no longer reacted blue to red litmus paper. By this modification the ammonia was liberated and only the nitrogen of the allantoïn was retained.

The flask and its contents are now left to cool and then the same flask, to avoid loss in transferring the contents to a Kjeldahl's combustion flask, is used for oxidation.

Pure sulphuric acid "free from nitrogen" is then added and the process continued as Kjeldahl's method by means of which the nitrogen is estimated and the allantoïn equivalent is calculated.

Deduction must be made for the nitrogen in the filter paper and the nitrogen in the pure sulphuric acid.

This modification has been tested with Merck's allantoïn, which itself has been found to be pure by Kjeldahl's method. .5 grm. was found, when dried in a desiccator, to yield .49 grm. The loss due to filtration at each stage of the test was calculated. This loss left .460 grm. to be recovered as the final filtrate was estimated as an aliquot portion. The actual recovery was .460 grm., or a complete recovery, i.e., 100%.

The urea nitrogen was estimated by Bohland's method.

From recent observations by Dr Noël Paton, but not yet published, show that the somewhat discredited method of Bohland, if properly carried out, gives results which correspond to within 4% with the more fashionable method of Mörner and Sjöqvist's.

The total nitrogen was estimated by Kjeldahl's method.

In each case the quantity of allantoïn in the urine was estimated.

In some cases the crystals were abundant

and visible in the urine before any chemical examination had taken place. This was illustrated in one case by the appearance of crystals to the amount of .39 grm. forming a deposit when 100 cc. of the urine were allowed to settle in a conical glass.

These crystals were examined under the microscope and found to be allantoïn.

Specimens were then mounted in glycerine jelly and are to be seen in the accompanying preparations.

Experiments

For the experiments the animals chosen were a setter and retriever bitch - each of known weight.

The allantoïn excretion was first found on a diet of oatmeal and milk.

Oatmeal contains .0212% purin nitrogen, and milk .002 grm. per litre according to Dr Walker Hall.[25] From this one finds that the diet of oatmeal porridge and milk contains .065 grm. purin nitrogen.

The nitrogenous balance with the above diet was then found to be fairly constant - the average being .549 grm. allantoïn in the urine of 24 hours. This average does not include the amount of allantoin excreted, with oatmeal diet, on the day following the administration of thymus gland, as the allantoïn had not been all eliminated within one day.

Having obtained the nitrogenous balance, the animals were fed upon a known weight of raw thymus gland.

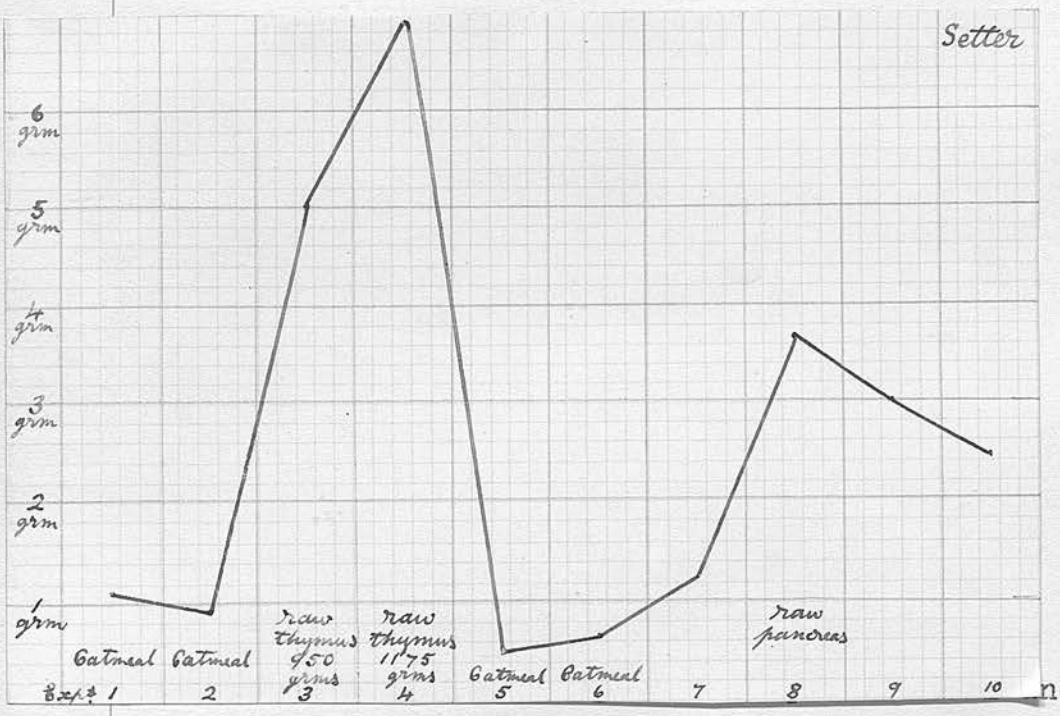
A marked rise in allantoïn excretion was the result.

The amount of allantoïn excreted then became 3.704 grm. per diem, or .708 grm. per 100 grms. thymus gland (excluding fat) in the case of the

retriever bitch.

With the setter the amount was 5.042 grm. per diem or .616 grm. per 100 grms. thymus gland.

This increase in allantoïn excretion may be illustrated by the chart.



nd milk.

To determine whether this increase is simply due to conversion of the nucleins and purins of the thymus gland or whether there may be some specific material in the thymus gland, the animals were next fed on boiled thymus gland with all the water in which it was boiled, retained. This contains all the nucleins and purins of the gland.

This diet shewed a rise in the amount of allantoïn excreted but it only amounted to 47% of the result obtained with the raw gland. Obviously

retriever bitch.

With the setter the amount was 5.042 grm. per diem or .616 grm. per 100 grms. thymus gland.

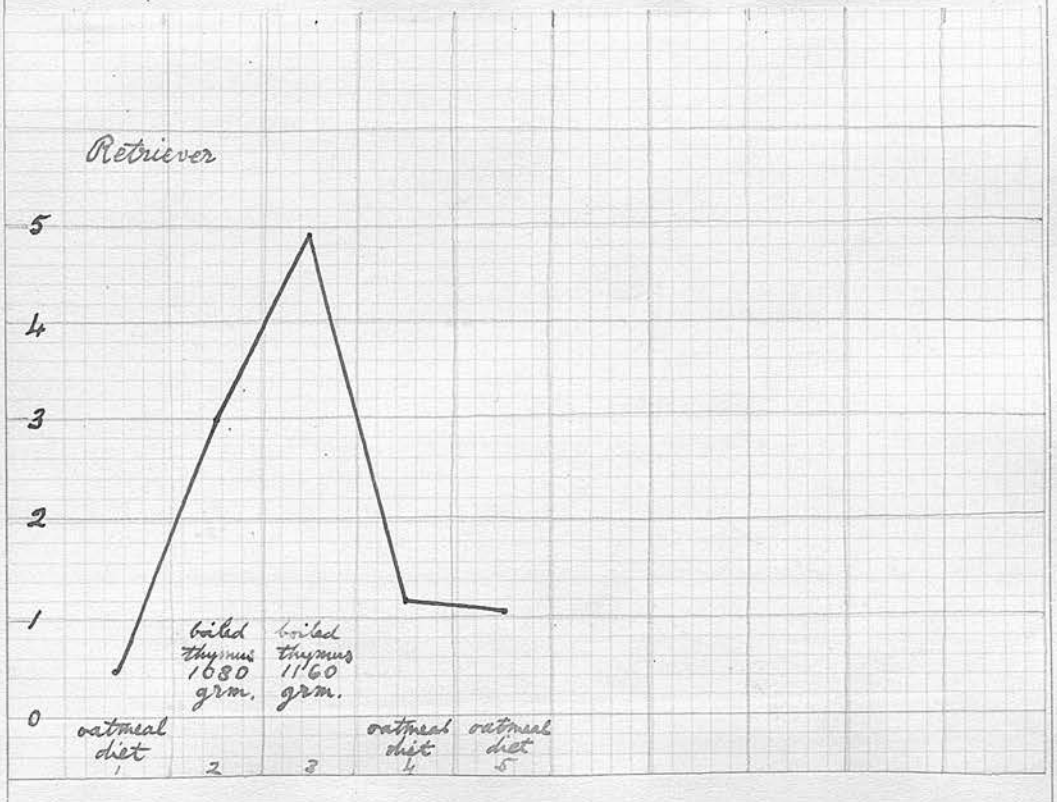
This increase in allantoïn excretion may be illustrated by the chart.

An almost equally abrupt fall was noticed when the animals were again fed upon oatmeal and milk.

To determine whether this increase is simply due to conversion of the nucleins and purins of the thymus gland or whether there may be some specific material in the thymus gland, the animals were next fed on boiled thymus gland with all the water in which it was boiled, retained. This contains all the nucleins and purins of the gland.

This diet shewed a rise in the amount of allantoïn excreted but it only amounted to 47% of the result obtained with the raw gland. Obviously

the boiled thymus although producing less effect than the raw gland contains something which is changed to allantoïn. Dr Walker Hall[26] has shewn that the thymus gland contains .4025% of purin nitrogen.

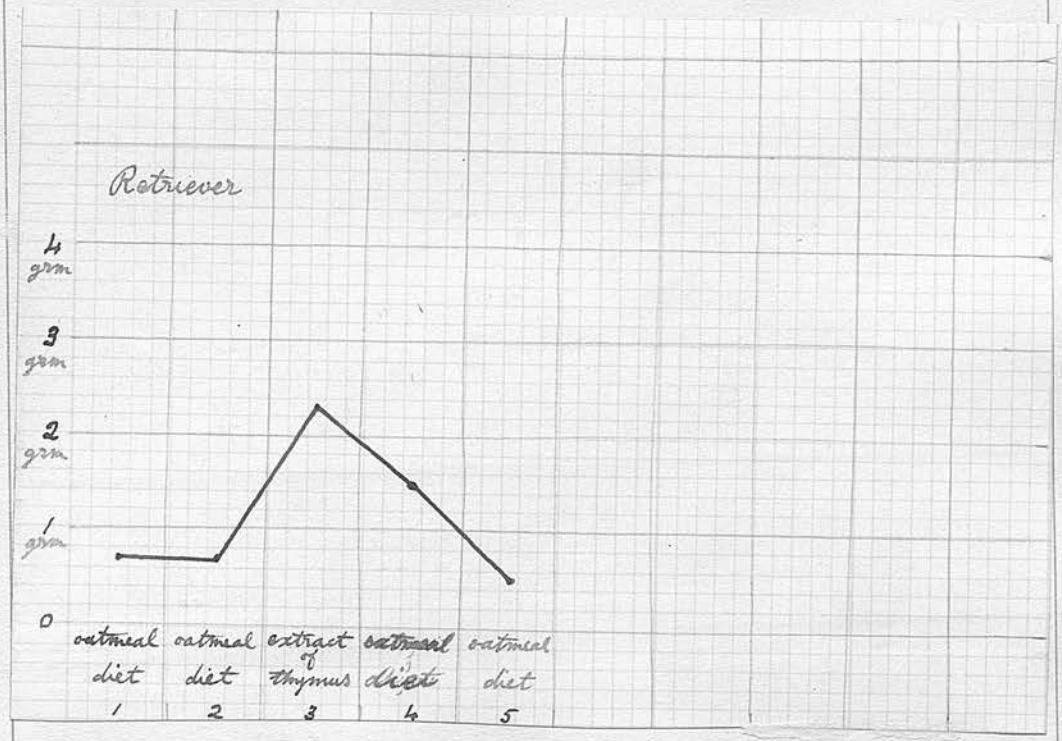


The results with boiled thymus gland would appear to indicate, from the above chart, a greater excretion of allantoïn than with the raw gland. This apparent increase over that of the raw gland is due to a larger amount of the boiled gland being administered.

To determine whether the nucleins of the gland or the purin bodies are the chief source, the dog was next fed on an "extract of thymus gland". This solution contains purins.

A rise in allantoïn excretion again takes

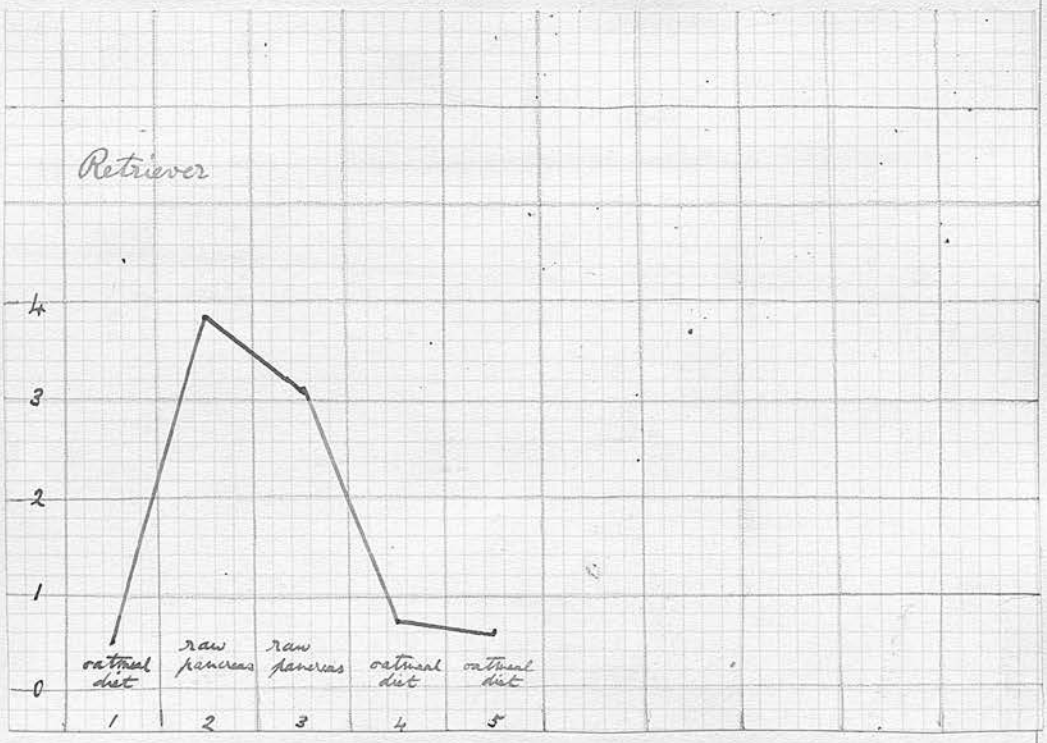
place, viz., 2.362 grm. per diem or .365 grm. per 100 grms. gland substance. This amounts to only 51% of the excretion obtained with the raw gland, but 4% higher than that with the boiled gland which contains the nucleins in addition to the purin bodies.



Hence it seems that the increase produced by boiled thymus gland, is due chiefly to the purins contained in it, since these are present to the same extent in boiled, as in raw thymus, and yet the yield of allantoïn is so much smaller.

In the experiments on feeding with raw pancreas, an increase of allantoïn was got but only amounting to 51% of that with the raw thymus gland, and the same as that with the extract of

thymus gland.



Time has not permitted me, as yet, to complete the experiments with boiled pancreas.

The following tables illustrate the above experiments.

Retriever bitch 15 kilos.

No.	Quantity	Sp. Gr.	Reaction	Allantoin total per diem	Allantoin per 100 grm gland — fat	Remarks
1	680cc	1020	alkaline	·473	—	300 grm oatmeal, 700cc milk 700cc water
2	470cc	1021	amphoteric	1·480	·251	684 grm. boiled thymus gland
3	380cc	1032	"	1·568	·267	680 grm. boiled thymus gland
4	360cc	1023	"	·727	—	oatmeal diet as above.
5	380cc	1022	"	—	—	"
6	600cc	1024	"	3·704	·708	680 grm raw thymus gland
7	182cc	1026	"	·524	—	oatmeal diet as above
8	930cc	1022	alkaline	·868	—	"
9	270cc	1022	amphoteric	·563	—	"
10	400cc	1043	"	3·848	·493	780 grm raw pancreas
11	570cc	1041	acid	3·102	·243	1071 grm. raw pancreas
12	530cc	1025	"	·789	—	oatmeal diet as above
13	605cc	1020	amphoteric	·818	—	"
14	670cc	1016	"	·597	—	"
15	570cc	1017	alkaline	·780	—	"
16	485cc	1020	"	·744	—	"

Retriever bitch (continued).

No.	Quantity	Sp. Gr.	Reaction	Allantoïn total per diem	Allantoïn per 100 grm gland — fat	Remarks
17	750cc	1015	alkaline	2.362	·365	extract of 750 grm thymus gland
18	840cc	1020	"	1·564	·241	oatmeal diet as above
19	620cc	1017	"	·477	—	"
20	650cc	1030	amphoteric	3·032	·325	1080 grm boiled thymus gland
21	1600cc	1020	alkaline	4·977	·498	1160 grm boiled thymus gland
22	675cc	1020	"	1·248	—	oatmeal diet as above
23	700cc	1015	"	1·120	—	"

Setter 14½ kilos

No.	Quantity	Sp. gr.	Reaction	Total N % in diluted urine	Total N per diem	Bohland N % in diluted urine	Bohland N per diem	Allantoïn % in diluted urine	Allantoïn per diem	Allantoïn per 100 grm gland-fat	Allantoïn N per diem % of total N	Remarks
1	1010cc	1017	acid	·423	6·348	·322	4·836	—	1·14	—	6·3	300grm oatmeal 700cc milk. 700cc water
2	870cc	1016	amphoteric	·465	5·210	·347	3·839	—	·901	—	6·1	"
3	190cc	1042	alkaline	·717	4·984	·412	2·863	·265	5·042	·616	35·8	950grm raw thymus
4	600cc	1049	acid	·643	14·159	·504	—	1·163	6·982	·690	21·2	"
5	725cc	1022	"	·552	7·176	—	—	—	·512	—	1·5	oatmeal diet as above
6	260cc	1025	"	·610	3·540	·456	2·644	—	·683	—	6·8	"
7	270cc	1033	amphoteric	·675	5·410	·489	3·918	·501	1·354	·130	13·2	1200grm raw pancreas
8	250cc	1053	acid	·706	9·178	·538	6·994	1·496	3·741	·361	14·3	—
9	510cc	1055	"	·748	19·448	·608	16·708	·596	3·042	·353	5·5	1000grm raw pancreas
10	290cc	1055	"	·692	11·072	—	—	—	2·480	·288	·9	oatmeal diet as above

So far as the experiments go they seem to indicate in the thymus gland there is something other than the nucleins and purins which plays a part in regulating the production of allantoïn. On this question it is hoped that further experiments will be conducted.

Summary.

1. Various methods of estimating allantoïn were tried and found defective, and a modification of Poduschka was finally devised which gave 100% recovery.
2. These observations shew that on a diet of oatmeal and milk yielding only about .065 grm. purin nitrogen, a dog passes from .512 grm. to 1.14 grm. allantoïn in the urine.
3. The feeding with raw thymus gland in dogs of about 15 kilos. gave a yield of from .6 to .7 grm. of allantoïn per 100 grms of fat-free thymus gland.
4. Feeding with boiled thymus gland gave from .2 to .4 grm. of allantoïn per 100 grms. of fat-free thymus gland.
5. Feeding with watery boiled extract of thymus gland gave .36 grm. per 100 grm of fat-free thymus gland.

6. Feeding with raw pancreas gave about from .37 to .4 grm. per 100 grm. of gland.

7. On Oatmeal and milk diet in one dog the nitrogen in allantoin was 6% of the total nitrogen and on a diet of raw thymus gland it rose to no less than 36%. On a larger amount of raw pancreas the proportion did not exceed 14% of the total nitrogen.

8. The close correspondence of the influence of boiled thymus with a thymus extract free from nucleins seems to indicate that it is the purins rather then the nucleins, which are the immediate forerunners of allantoïn.

9. The fact that boiling thymus decreases its effect on the production of allantoïn by nearly 50%, seems to show that the influence of the food is not entirely due to the nucleins or purins contained, but that the thymus must have some specific action on the metabolism leading to the production of allantoïn and to its non-conversion to urea.

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Purin Bodies pp. 46 and 49

26. I. Walker Hall

Purin Bodies p. 41.

Appendix

As previously mentioned the modification of Poduschka recovers 100% of the allantoïn.

The following example illustrates this:-

.5 grm. allantoïn (Mercks) when dried in a desiccator until of constant weight yielded .49 grm. This was dissolved in 640 cc. water and made up to 641 cc. with basic lead acetate and filtered. The filtrate measured 618 cc. or a loss of 23 cc in 641, i.e., a loss of 22.9 cc. on the original 640 cc.

This quantity (618 cc.) was then made up to 619 cc. with sodium sulphate and filtered when it was found to yield only 608 cc. or a loss of 11 cc. on 619 cc., i.e, a loss of 10.9 cc. on the original amount at this stage, viz., 618 cc.

This 608 cc. was made up to 628 cc. with silver nitrate solution and filtered yielding 625 cc. or a loss of 3 cc. on 628 cc., i.e., a loss of 2.9 cc. on 608 cc.

The combined loss in filtration is equal to 36.7 of 640 cc. original solution.

One has therefore to recover 603.3 cc. But 603.3 cc. must be equal to only .460 grm. allantoïn since 640 cc. contain .49 grm.

.460 grm. of allantoïn remain to be recovered.

150 cc. $\frac{N}{10}$ acid

32 cc. $\frac{N}{10}$ soda

118 cc.

1.4

472

118

165.2 mgr.

.1652 grm.

.00196 N. in filter paper + H_2SO_4

.16324

56N: .16324N::158 grm allantoïn

158

130592

81620

16324

56 } 8 ⟌25.79192

7⟌3.22399

.460 grm allantoïn recovered from .460 grm. to be recovered, i.e., 100% recovered.

With unknown quantities one estimates the amount in the final filtrate and calculates by simple proportion (having already measured the loss) what would have been in the filtrate had there been no loss in filtration, i.e., one treats the final filtrate as an aliquot portion.